

DECLARATION FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, WE HEREBY DECLARE THAT:

Our residence, post office address, and citizenship are as stated below next to our names.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: METHODS AND COMPOSITIONS TO MODULATE EXPRESSION IN PLANTS, the specification of which is attached hereto unless the following box is checked:

☒ was filed on January 19, 2001, as United States Application Serial No. 09/765,555.

WE HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

We acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

We hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed?
*			<input type="checkbox"/> Yes <input type="checkbox"/> No

We hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
60/327,552	July 21, 2000
60/177,468	January 21, 2000

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6/13/3
Date

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Post Office Address: 7353 Mannix Court, San Diego, California 92129

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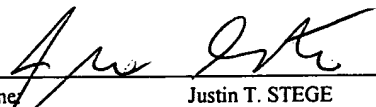
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Date	Name:	Carlos F. BARBAS, III
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6/14/03		
Date	Name:	Justin T. STEGE
	Residence:	San Diego, California
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Date	Name:	Xueni GUAN
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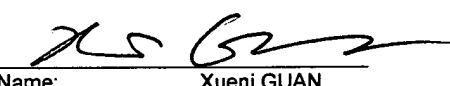
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We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Plant Biotechnology

Authors: Randy Vines, Extension Specialist, Biotechnology Information; Virginia Tech

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Biotechnology and Plants

Today, biotechnology is being used as a tool to give plants new traits that benefit agricultural production, the environment, and human nutrition and health. The purpose of this publication is to provide basic information about plant biotechnology and to give examples of its uses

The goal of plant breeding is to combine desirable traits from different varieties of plants to produce plants of superior quality. This approach to improving crop production has been very successful over the years. For example, it would be beneficial to cross a tomato plant that bears sweeter fruit with one that exhibits increased disease resistance. To do this, it takes many years of crossing and backcrossing generations of plants to obtain the desired trait. Along the way, undesirable traits may be manifested in the plants because there is no way to select for one trait without affecting others. Another limitation of traditional plant selection is that breeding is

restricted to plants that can sexually mate.

Advances in scientific discovery and laboratory techniques during the last half of the twentieth century led to the ability to manipulate the deoxyribonucleic acid (DNA) of organisms, which accelerated the process of plant improvement through the use of biotechnology.

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The Science of Modern Plant Biotechnology

Genes and the Genome

Plants are made of millions of cells all working together. Every cell of a plant has a complete "instruction manual" or genome (pronounced "JEE-nom") that is inherited from the parents of the plant as a combination of their genomes.

Genes are found within the genome and serve as the "words" of the instruction manual. When a cell reads a word, or in scientific terms "expresses a gene," a specific protein is produced. Proteins give an individual cell, and therefore the plant, its form and function. Genes (words) are written using the four-letter alphabet A, C, G, T. The letters are abbreviations for four chemicals called bases, which together make up DNA. DNA is universal in nature, meaning that the four chemical bases of DNA are the same in all living organisms. Consequently, a gene from one organism can function in any other organism.

The ability to move genes into plants from other organisms, thereby producing new proteins in the plant, has resulted in significant achievements in plant biotechnology that were not possible using traditional breeding practices.

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Methods of Introducing Genes into Plants

To genetically modify a plant, the thousands of bases of DNA comprising an individual gene are transferred into an individual plant cell where the new gene becomes a permanent part of the cell's genome. This process makes the resulting plant "transgenic." Transfer of DNA into plant cells is done using various "transformation" techniques that are the result of discoveries in basic science.

Nature's way

One method to transfer DNA into plants takes advantage of a system found in nature. The bacterium that causes "crown gall tumors" injects its DNA into a plant genome, forcing the plant to create a suitable environment for the bacterium to live. After discovering this process, scientists were able to "disarm" the bacterium, put new genes into it, and use the bacterium to harmlessly insert the desired genes into the plant genome.

Cellular target practice

In the "biolistic" or "gene gun" method, microscopic gold beads are coated with the gene of interest and shot into the plant cell with a burst of helium. Once inside the cell, the gene comes off the bead and integrates into the cell's genome.

That's shocking!

It was also discovered that plant cells could be "electroporated" or mixed with a gene and "shocked" with a pulse of electricity, causing holes to form in the cell through which the DNA could flow. The cell is subsequently able to repair the holes and the gene becomes a part of the plant genome.

Selecting the right cells

When using these methods, new genes are successfully introduced into only a small percentage of the cells, so scientists must be able to "pick out" or "select" the transformed cells before proceeding. This is often done by concurrently introducing an additional gene into the cell that will make it resistant to an antibiotic. A cell that survives antibiotic treatment will most likely have received the gene of interest as well; that cell is subsequently used to propagate the new plant. There is a concern that the gene giving antibiotic resistance could naturally be transferred to bacteria once the transgenic plant is in the wild, making bacteria resistant to antibiotics that are used to fight human infection. Scientists are currently devising ways to select for transformed cells that will alleviate this issue.

Timeline of Plant Biotechnology

1700s -- Naturalists identify hybrid plants

1860s -- Austrian botanist and monk Gregor Mendel studies pea plants and recognizes that specific traits are passed from parents to offspring - these traits are eventually discovered to be genes

1900 -- European botanists begin to improve plant productivity using genetic theories based on Mendel's work

1922 -- Farmers purchase hybrid seed corn created by crossbreeding two corn varieties

1953 -- Structure of DNA is discovered - marking the beginning of modern genetic research

- 1970s -- Hybrid seeds are introduced to developing countries to increase food supplies
- 1973 -- Genetic engineering is used to precisely manipulate bacterial DNA
- 1983 -- First GM plant is created; a tobacco plant resistant to an antibiotic
- 1985 -- GM plants resistant to viruses, bacteria, and insects are field tested
- 1986 -- EPA approves the release of the first GM crop (herbicide resistant tobacco)
- 1990 -- First successful field trial of GM cotton (herbicide resistant)
- 1992 -- FDA decides GM foods will be regulated as conventional foods
- 1994 -- FlavrSavr Tomato becomes the first GM food to be approved for sale
- 1995 -- Herbicide resistant canola, corn,
- 2000 -- cotton, soybeans, sugar beet as well as insect or virus resistant corn, cotton, papaya, potato, squash, tomato approved in the U.S.
- 2001 -- "Golden rice" which may help prevent millions of cases of blindness and death caused by Vitamin A and iron deficiencies undergoes continued testing

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Traits Being Introduced Into Plants

Changes made to plants through the use of biotechnology can be categorized into the three broad areas of input, output, and value-added traits. Examples of each are described below.

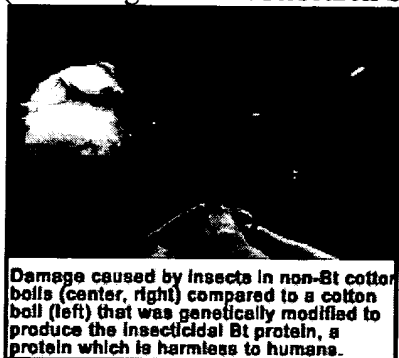
Input traits

An "input" trait helps producers by lowering the cost of production, improving crop yields, and reducing the level of chemicals required for the control of insects, diseases, and weeds.

Input traits that are commercially available or being tested in plants:

- Resistance to destruction by insects
- Tolerance to broad-spectrum herbicides
- Resistance to diseases caused by viruses, bacteria, fungi, and worms
- Protection from environmental stresses such as heat, cold, drought, and high salt concentration

(credit: Agricultural Research Service, USDA)



Damage caused by insects in non-Bt cotton bolls (center, right) compared to a cotton boll (left) that was genetically modified to produce the insecticidal Bt protein, a protein which is harmless to humans.

Output Traits

An "output" trait helps consumers by enhancing the quality of the food and fiber products they use.

Output traits that consumers may one day be able to take advantage of:

Nutritionally enhanced foods that contain more starch or protein, more vitamins, more anti-oxidants (to reduce the risk of certain cancers), and fewer trans-fatty acids (to lower the risk of heart disease)

Foods with improved taste, increased shelf-life, and better ripening characteristics

- Trees that make it possible to produce paper with less environmental damage
- Nicotine-free tobacco
- Ornamental flowers with new colors, fragrances, and increased longevity

"Value-added" traits

Genes are being placed into plants that completely change the way they are used.

Plants may be used as "manufacturing facilities" to inexpensively produce large quantities of materials including:

- Therapeutic proteins for disease treatment and vaccination
- Textile fibers
- Biodegradable plastics
- Oils for use in paints, detergents, and lubricants

Plants are being produced with entirely new functions that enable them to do things such as:

- Detect and/or dispose of environmental contaminants like mercury, lead, and petroleum products

Canola Plants made Resistant to High Concentrations of Salt Through Biotechnology

(credit: Dr. Eduardo Blumwald, University of California, Davis)



Canola plants grown in the presence of a high concentration of salt. Non-genetically modified canola (non-GM) or canola genetically modified to have high, medium, or low tolerance to salt.

Plants with "input traits" that are commercially available include:

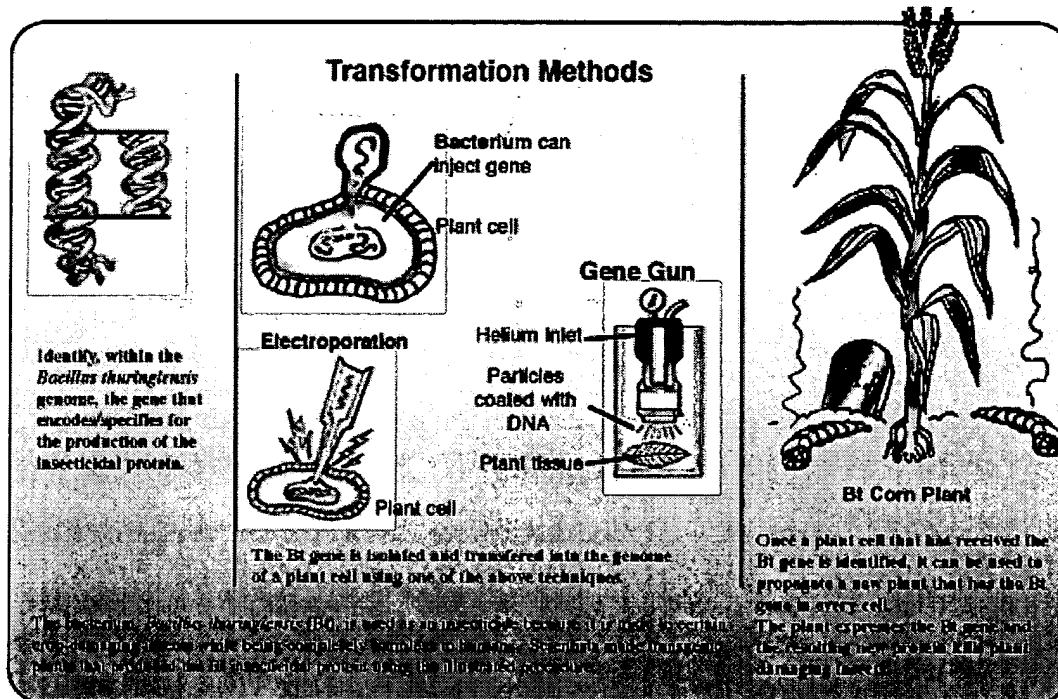
- Roundup Ready® soybean, canola, and corn: resistant to treatment with Roundup herbicide that may result in more effective weed control with less tillage, and/or decreased use of other, more harmful herbicides
- YieldGard® corn and Bollgard® cotton: express an insecticidal protein that is not toxic to animals or humans which protects the plant from damage caused by the European corn borer, tobacco budworm, and bollworm
- Destiny III® and Liberator III® squash: resistant to some viruses that destroy squash

Plants may become available with "output traits" including:

- High laurate canola and high oleic soybean having altered oil content to be used primarily in industrial oils and fluids rather than food
- High-starch potatoes that take up less oil when frying
- Longer shelf-life bananas, peppers, pineapples, strawberries, and tomatoes
- Soybeans with higher levels of isoflavones; compounds that may be beneficial in reducing some cancers and heart disease
- Plants that produce vaccines and pharmaceuticals for treatment of human diseases

- Corn with improved digestibility and more nutrients providing livestock with better feed

How to Make a Plant that Produces its own Insecticide



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Issues Associated with Genetically Modified Plants

Benefits and Risks

The list of plants and plant-derived products made as a result of modern biotechnology is ever-increasing. Many transgenic plants, such as herbicide resistant soybeans, have been widely adopted by producers signifying their satisfaction, while other products, such as the delayed softening "FlavrSavr" tomato, are no longer on the market.

Some of the potential benefits from using transgenic plants include:

- Reduced crop production costs and increased yields
- Healthier, more nutritious foods
- Reduced environmental impact from farming and industry
- Increased food availability for underdeveloped countries

Potential risks associated with transgenic plants include:

- Introduction of allergenic or otherwise harmful proteins into foods
- Transfer of transgenic properties to viruses, bacteria, or other plants
- Detrimental effects on non-target species and the environment

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Safety, Regulation, and Labeling

In the United States

At the Federal level, the Food and Drug Administration, the Environmental Protection Agency, and the Department of Agriculture extensively review products of biotechnology to ensure that they are safe for public use and the environment.

GM foods require labeling only if they differ significantly in safety, composition, or nutritional content when compared to their non-GM counterparts. Additionally, the FDA requires a GM food to be labeled if it contains a known allergen unless data have shown that there is no allergy risk.




In Organic products

Organic standards reflect a "zero tolerance" policy concerning transgenic products and organisms. Organic food producers are taking precautions to minimize the risk of unintentional contamination of their products with transgenic ones.

In Canada

The Canadian Food Inspection Agency, Health Canada, and Environment Canada strictly regulate agricultural biotechnology products. They currently require GM foods to be labeled if they differ

significantly in composition or nutritional value and support a voluntary labeling policy for others.





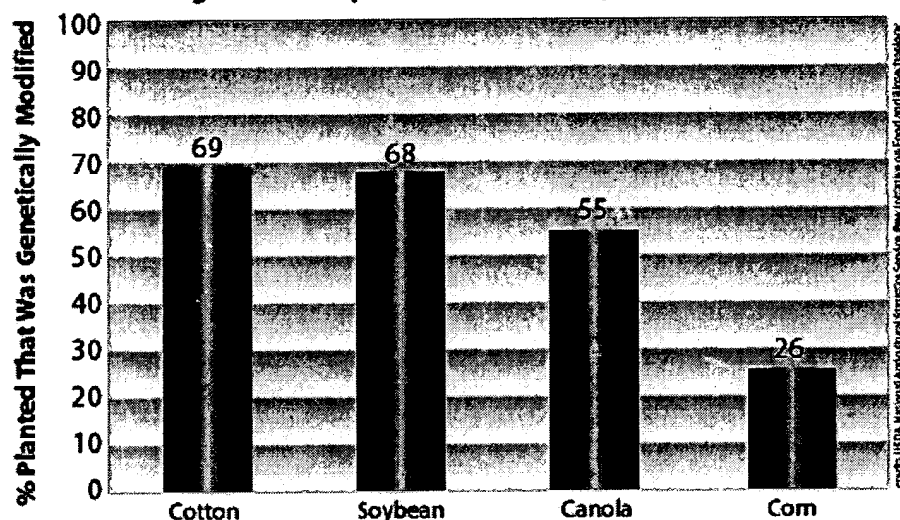
In Europe

The acceptance of GM crops by the European Union has been more reserved. However, recent statements made by European Union officials suggest that their position may be changing as they are calling for their policies regarding GM

crops to be based on scientific principles rather than on public opinion and misconceptions. Europe currently favors labeling of all GM foods and a system that would allow for "identity preserved" processing in which foods would be guaranteed to contain no genetically modified products.

(credit: USDA National Agricultural Statistics Service, Pew Initiative on Food and Biotechnology)

Percentage of U.S. Crop that was Genetically Modified (2001)



In 2001, U.S. farmers planted an increased amount of crops that were genetically modified to be resistant to pests and herbicides.

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